Journal of Experimental Marine Biology and Ecology 514–515 (2019) 10–17

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Evidence for different thermal ecotypes in range centre and trailing edge kelp populations

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ARTICLE INFO

Keywords:
Laminaria digitata
Range-centre
Trailing-edge
Climate change
Heat shock

ABSTRACT

Determining and predicting species' responses to climate change is a fundamental goal of contemporary ecology. When interpreting responses to warming species are often treated as a single physiological unit with a single species-wide thermal niche. This assumes that trailing edge populations are most vulnerable to warming, as it is here where a species' thermal niche will be exceeded first. Local adaptation can, however, result in narrower thermal tolerance limits for local populations, so that similar relative increases in temperature can exceed local niches throughout a species range. We used a combination of common garden temperature heat-shock experiments (8–32 °C) and population genetics (microsatellites) to identify thermal ecotypes of northeast Atlantic range centre and trailing edge populations of the habitat-forming kelp, *Laminaria digitata*. Using upregulation of *hsp70* as an indicator of thermal stress, we found that trailing edge populations were better equipped to tolerate acute temperature shocks. This pattern was consistent across seasons, indicating that between-population variability is fixed. High genetic structuring was also observed, with range centre and trailing edge populations representing highly distinct clusters with little gene flow between regions. Taken together, this suggests the presence of distinct thermal ecotypes for *L. digitata,* which may mean responses to future warming are more complex than linear range contractions.

1. Introduction

Temperature is one of the most important drivers of ecological patterns and processes (Hutchins, 1947), dictating where a species can exist and how well it performs throughout its distribution (Brown, 1984; Dunson and Travis, 1991; Gaston, 2003). Rising global temperatures, from anthropogenic greenhouse gas emissions, have already resulted in the altered performance and poleward range migrations of a range of biota and is set to continue as climate change advances (Walther et al., 2002; Parmesan and Yohe, 2003; Burrows et al., 2011; Sunday et al., 2012; Poloczanska et al., 2013). As range migrations and altered performances can have serious implications for the structure and functioning of entire ecosystems (Walther et al., 2002, Parmesan, 2006, Doney et al., 2011), understanding the effect of rising temperatures on species performance and distributions is a key goal in climate change ecology. However, if this is to be achieved then we must first understand the physiological traits and mechanisms that govern existing species distributions.

When forecasting future distributions, species are often treated as a single homogenous unit (Pearman et al., 2010; Reed et al., 2011), with populations assumed to exhibit similar thermal limits throughout the species' range. Therefore, thermal safety margins, the buffer between experienced temperatures and a species upper thermal limits (see Deutsch et al., 2008; Bennett et al., 2015), are assumed greatest at the range centre and lowest at trailing edges. As such, range centre populations are generally considered to be less vulnerable to predicted warming trends than trailing edge populations, where thermal safety margins will be exceeded first (Thomas et al., 2006; Thomas, 2010). Along temperature gradients, however, thermal tolerances are not always consistent between populations, as local adaptation and/or phenotypic plasticity can result in thermal limits being different across a species biogeographic range (Sanford and Kelly, 2011). This may result

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https://doi.org/10.1016/j.jembe.2019.03.004
Received 9 November 2018; Received in revised form 4 February 2019; Accepted 14 March 2019
Available online 21 March 2019
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in more complex responses to warming than simple linear range contractions from trailing edges (Sanford and Kelly, 2011; Valladares et al., 2014; Bennett et al., 2015; Pontes-da-Silva et al., 2018), meaning central populations may also be vulnerable to ongoing warming.

Within the context of decadal scale warming, the significance of intraspecific variation in thermal niche largely depends on the mechanisms responsible (i.e. plasticity vs. adaptation). If the response is plastic then species are likely to be able to keep pace with climate warming, but if responses are a result of adaptation then the pace of warming is likely to be too fast for natural selection (Jump and Penuelas, 2005; Quintero and Wiens, 2013). Gaining an understanding of gene flow can provide valuable insight into whether adaptation or plasticity is favoured. Where gene flow is greater than the selection gradient, there is likely to be little selection for local thermal adaptation, resulting in a single plastic phenotype (García-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997), whereas restricted gene flow facilitates the development of local ecotypes (Endler, 1977).

Sessile organisms, that cannot modify their behaviour, rely on physiological mechanisms, underpinned by modulation of gene expression, to mediate periods of thermal stress. Whilst the mechanisms themselves are evolutionary conserved, patterns in gene expression are heritable and can vary considerably between populations (López-Maury et al., 2008) resulting in population level differences in thermal tolerance (e.g. Henkel and Hofmann, 2008). Therefore, analysis of variation in gene expression offers a powerful tool to identify thermal set points of a population that are often apparent before higher level physiological differences are observed. The Heat Shock Response (HSR) is perhaps the most well studied mechanism for identifying differences in thermal physiology. When organisms are challenged by elevated temperatures that result in protein denaturation and aggregation they rapidly upregulate a suite of molecular chaperones known as heat shock proteins (HSPs). These HSPs preserve normal cell function by ensuring appropriate protein folding during translation (Frydman, 2001), membrane stability and transport (Hartl and Hayer-Hartl, 2002) and protein refolding (Hendrick and Hartl, 1993).

Kelps are large brown habitat-forming seaweeds that form extensive forests along rocky coastlines in temperate and subpolar regions (Steneck et al., 2002; Smale et al., 2013; Teagle et al., 2017). Kelp forests rank among the world's most productive and extensive habitats, being distributed along one-quarter of the world's coastlines and rivalling the productivity of tropical rainforests (Leith and Whittaker, 1975; Mann, 1973). Kelp exist over vast temperature gradients and whilst long distance dispersal is possible (e.g. Fraser et al., 2018) the majority of spores settle within a few meters of the parent alga (e.g. Norton, 1992; Kendrick and Walker, 1995). Therefore, local adaptation may be a common feature throughout kelp distributions. Given that climate mediated range shifts have already been observed in kelp forests across the world (Marba and Duarte, 2010; Wernberg et al., 2016; Krumhansl et al., 2016) and are predicted to continue as warming progresses (e.g. Martínez et al., 2012; Jueterbock et al., 2013; Khan et al., 2018; Assis et al., 2018) understanding whether intraspecific variation in thermal niche may make central populations vulnerable to future warming trends has direct relevance for management and conservation of kelp forest ecosystems.

In this study we investigated intraspecific variation in the Heat Shock Response (HSR) of *Laminaria digitata* (Hudson) J.V. Lamouroux, a common transatlantic kelp. Specifically, we compared populations from two thermally distinct regions in the United Kingdom (representing East Atlantic range centre and trailing edge populations) and conducted experiments at the coolest and warmest times of year, to characterise intraspecific and intra-annual variability in HSRs. We also used neutral microsatellite markers to gain an understanding of gene flow between populations. By adopting a multi-pronged experimental approach we aimed to determine (i) whether populations show differentiation in thermal niche and (ii) whether such differences are likely a product of plasticity or adaptation.

2. Methods

2.1. Study species

L. digitata is an important ecosystem engineer that forms highly productive stands supporting rich associated communities (Schultze et al., 1990). It has a transatlantic-boreal distribution stretching from the Arctic, where sea ice cover, light availability and low temperatures limit its leading edge, to the English Channel in the East Atlantic and Cape Cod along the US Eastern Seaboard, where it is limited by high summer temperatures. Currently, no climate related population declines have been observed in the West Atlantic (Merzouk and Johnson, 2011) but declines in abundance have been reported in the East Atlantic, although disentangling the roles of overexploitation and warming is difficult (Cosson, 1999; Simkanin et al., 2005; Gevaert et al., 2008). However, as climate change advances, population losses are predicted at both *L. digitata's* East and West Atlantic trailing edges (Raybaud et al., 2013; Khan et al., 2018). In the West Atlantic a lack of subtidal competition means *L. digitata* can extend well into the subtidal (> 20 m) but in the East Atlantic the presence of the subtidal congener *L. hyperborea* restricts *L. digitata* to the low intertidal/shallow subtidal. This means that East Atlantic populations will not be able to move to deeper locations as temperatures in the intertidal become inhospitable. However, the narrow linear distribution of *L. digitata*'s East Atlantic range make it ideally suited to understanding how temperature facilitates the development of thermal ecotypes as it is unconfounded by covarying factors associated with depth.

2.2. Heat shock experiment

2.2.1. Patterns of the heat shock response

One of the most highly conserved and well-studied group of HSPs is the Hsp70 family. By measuring the thermal profile of *hsp70* upregulation (temperature at which HSPs are synthesised, T_{on}, maximally expressed, T_{peak}, and turned off, T_{off}) it is possible to investigate intra and inter specific differences in thermal tolerance (Tomanek and Somero, 1999; Barua and Heckathorn, 2004; Tomanek, 2010). T_{on} represents the minimum temperature to cause stress, T_{peak} the upper thermal limit of tolerance beyond which protein synthesis can no longer meet the cellular response demands and T_{off} the likely ultimate upper limit in the functioning of the translational machinery (Barua and Heckathorn, 2004; Tomanek, 2010). By comparing these profiles between populations at different latitudes within a species' biogeographic range, it is possible to quantify intraspecific variation in thermal tolerances and to begin to predict how populations, and species, may respond to continued warming.

2.2.2. Survey periods and sites

We sampled *Laminaria digitata* populations by employing a nested hierarchical design. Two regions were selected to represent range centre and trailing edge populations (Fig. 1); Within each region two sampling sites were randomly selected; Warbeth, northern Scotland (hereafter 'range centre 1'), Easdale western Scotland (hereafter 'range centre 2'), Trevone, southwest England (hereafter 'trailing edge 1') and St Mawes, southwest England (hereafter 'trailing edge 2'). These regions are thermally distinct with a mean temperature difference of ~ 2.5 °C in winter and ~ 4 °C in summer (Smale and Moore, 2017; Pessarrodona et al., 2018). Although the absolute range edge of *L. digitata* extends to Brittany, (~ 48°N), the temperature regimes either side of the English Channel are similar (mean September SST near St Mawes – 'trailing edge 2 = 17 °C, mean SST in southern Brittany, France = 17.4 °C), and as such, the southwest England sites sampled here were considered to support representatives of the wider marginal range-edge population (King et al., 2018a). SST were obtained from the United States' National Oceanographic and Atmospheric Administration (NOAA) daily satellite readings (http://www.seatemperature.org).

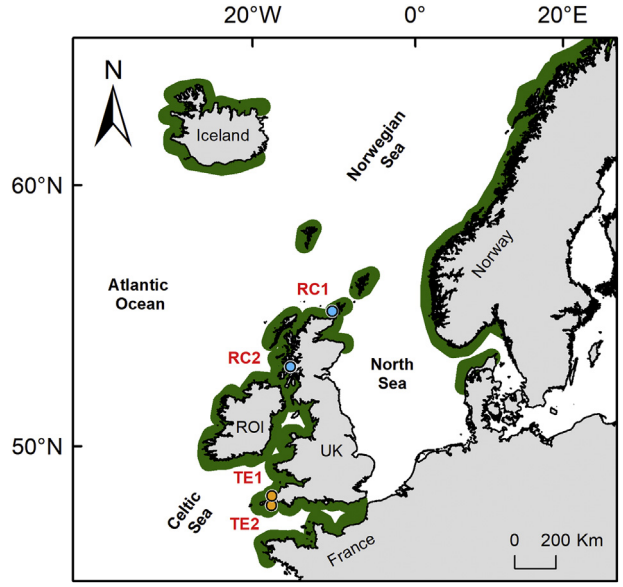

Fig. 1. Sampling sites for *Laminaria digitata*. Range centre 1 (RC1) = Warbeth Bay, Orkney. Range centre 2 (RC2) = Easdale, west-Scotland. Trailing edge 1 (TE1) = Trevone, Cornwall. Trailing edge 2 (TE2) = St Mawes, Cornwall. Shaded area = approximate distribution of *L. digitata*.

2.2.3. Sample collection and thermal assays

To investigate the plasticity of the HSR, the experiment was conducted across two sampling periods, spring (April) and summer (September), representing thermal minima and maxima in ambient seawater temperature (Smale and Moore, *unpublished*). The sampling periods also varied in the intensity of recent low tide heat shock events experienced, with cooler aerial temperatures experienced over the winter and early spring compared to higher aerial temperatures and sunshine hours during the summer. Thermal assays were conducted for all sites in spring but in summer, it was only possible to conduct assays for range centre 1 and trailing edge 1.

At each site, five mature sporophytes were collected during low spring tides from within the *L. digitata* zone. Individuals were brought back from the field in cool dark containers and held in aerated recirculating tanks for 7–14 days under photosynthetic photon flux density of ~20 μmol m^{-2} s^{-1} (12:12 h light: dark cycle). Acclimation tanks were maintained at 8 °C in spring and 12 °C in summer, representing the lowest monthly mean temperature experienced in the field by any population for that month (i.e. range centre 1) (Smale and Moore, *unpublished*). This acclimation period was necessary to ensure all individuals were not physiologically stressed prior to the thermal assays.

Thermal stress assays were conducted on discs of tissue (27 mm diameter, area of 11.45 mm^2) excised from each kelp using a cork borer. Such an approach is representative of the greater organismal response and is well established in seaweed gene expression studies (e.g. Henkel and Hofmann, 2008; Pearson et al., 2009; Jueterbock et al., 2014; King et al., 2018a). Each disc was heat shocked at one of seven temperatures (8, 12, 16, 20, 24, 28 or 32 °C) for one hour in thermostatically controlled water baths with recirculating, aerated seawater. After one hour's heat shock, discs were removed, blotted dry, snap frozen in liquid N_2 and stored at −80 °C until RNA extraction.

2.2.4. RNA extraction and qPCR

Total RNA was extracted following a protocol from Pearson et al. (2006). Primers and qPCR conditions for *hsp70* and two reference genes (18 s ribosomal RNA and Rubisco large sub unit, Table S1) followed that of King et al. (2018a). Relative mRNA levels were calculated as follows: Firstly, the difference in Ct values from *hsp70* and the internal reference gene were calculated (ΔCT). The ΔCT value was then subtracted by the ΔCT of the control (8 °C) from each individual (ΔΔCT). Relative expression of *hsp70* was then calculated by $e^{(-\Delta\Delta CT)}$.

2.2.5. Defining thermal set points

The thermal set points were defined as follows: $T_{(on)}$; the temperature at which upregulation first becomes evident, T_{peak}; the temperature of maximal expression and $T_{(off)}$; the temperature post T_{peak} where there is an obvious decline in expression, indicating the HSR has been turned off.

2.2.6. Statistical analysis

2.2.6.1. Regional comparison. Differences in upregulation of *hsp70* to heat stress between regions was assessed using univariate permutational ANOVA, using the PERMANOVA module (Anderson, 2001) within Primer 6 software (Clarke and Gorley, 2001). The experiment conducted in spring was first analysed in isolation to examine site and region-level variability. Here, the model included three factors; region (fixed factor; 2 levels – range centre and trailing edge), site (random factor; 2 sites nested within each region) and temperature (fixed factor; 7 levels - 8, 12, 16, 20, 24, 28 & 32 °C).

2.2.6.2. Seasonal Comparison. When comparing expression between regions and seasons only trailing edge 1 and range centre 1 were analysed. The model had three factors: region (fixed factor; 2 levels – range centre and trailing edge), season (fixed factor; 2 levels – spring and autumn) and temperature (fixed factor; 5 levels - 16, 20, 24, 28 & 32 °C). As expression values of *hsp70* were normalised to ambient sea temperatures for the time of year sampled (8 °C in spring and 12 °C in autumn) it was not possible to directly compare all temperatures between seasons. Instead, temperatures 16, 20, 24, 28 & 32 °C were used for the formal analysis between seasons.

Permutations (9999 under a reduced model) were conducted on a similarity matrix constructed from Euclidean distances between untransformed data (relative hsp70 expressions) for each sampling period. When conducting PERMANOVA analysis on univariate data using Euclidean distances, outputs (F-statistics) are analogous to traditional least-square ANOVA, without the same severity of assumptions regarding data distributions and homogeneity of variance (Anderson, 2001; McArdle and Anderson, 2001).

2.3. Population genetics

2.3.1. Sample collection

Thirty individuals, at least 5 m apart from one another, were haphazardly sampled at each site by excising fresh tissue from directly above the meristem between May – June 2016. Samples were then dried in individual Eppendorf's with silica drying crystals until DNA extraction.

2.3.2. DNA extraction and microsatellite amplification

Genomic DNA was isolated from 5 to 10 mg of dried tissue ground to a fine powder using a ball mill. 500 μl of extraction buffer (100 mM Tris, 25 mM EDTA, 1.4 M NaCl, 1% PVP and 2% CTAB, pH 8) was added and vortexed and left at room temperature (RT) for 10 min. RNA was digested by adding 2 μl RNAse A (10 mg/ml) to the solution and incubating at 55 °C for one hour. Total DNA was extracted by chloroform extraction, 500 μl of chloroform:Isoamyl alcohol (24:1 *v*/v) was added, vortexed vigorously and centrifuged at 14000*g* for 10 min and the upper aqueous layer transferred to a new tube. DNA was precipitated out with 30 μl of ammonium acetate and 200 μl isopropanol at −20 °C for 30 min. Samples were centrifuged at 14000*g* for 10 min at RT. Two EtoH washes were performed at 70% and 95% and recollected at 14000 g for 10 min at RT. Pellets were air dried for one hour and then resuspended in 50 μl DEPC-treated water.

Twelve microsatellite markers previously developed for *Laminaria digitata* (Ld148, Ld158, Ld167, Ld371, Ld531 and Ld704) and *Laminaria*

ochroleuca (Lo4-24, Lo454-17, Lo454-23, Lo454-24, Lo454-27, and Lo454-28) were used (Robuchon et al., 2014). Microsatellites were amplified by individual PCR in 10 µl final volumes containing 1 X GoTaq Flexi colourless reaction buffer, 2 mM $MgCl_2$, 150 µm dNTPs, 0.35 U GoTaq DNA polymerase (Promega) and 2 µl template (1:50 dilution) following the protocol of Robuchon et al. (2014). For Ld-primers PCR cycling parameters consisted of an initial denaturation at 94 °C for 5 min, five cycles of touchdown PCR (denaturation at 94 °C for 45 s, annealing at 60 °C for 1 min and extension at 72 °C for 45 s; 1 °C decrease of annealing temperature every cycle) followed 50 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 1 min and a final extension at 72 °C for 30 min. For Lo-primers PCR cycling parameters consisted of an initial denaturation at 94 °C for 5 min, 5 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 45 s followed by 30 cycles where all conditions were similar apart from annealing temperature which was changed to 55 °C. A final extension of 72 °C for 20 min was then performed. Forward primers were fluorescently labelled with VIC (Ld167 and Lo454-17), PET (Ld148, Ld371, Lo454-23 and Lo454-28), NED (Ld158, Ld371, Lo454-23 and Lo454-28) or FAM (Ld531 and Lo454-24) dye (Eurogentec). Amplicon fragment size was analysed on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems) and alleles were scored manually using PEAKSCANNER software v 1.0.

2.3.3. *Estimating genetic structure - statistical analysis*

Genetic variation within samples was characterised using number of alleles (NA), allelic richness (AR), observed heterozygosity (HO), and expected heterozygosity (HE) all calculated using FSTAT 2.9.3 (Goudet, 1995). Mean pairwise relatedness within samples was calculated using the relatedness estimator, rqg, of Queller and Goodnight (1989) in GENALEX 6.2 (Peakall and Smouse, 2006) with associated 95% confidence intervals determined by 1000 bootstraps. Permutation of genotypes among all samples (999 times) was used to calculate the upper and lower 95% confidence intervals for the expected range of rqg under a panmictic model. Genotype frequency conformance to Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium between pairs of loci were tested using exact tests (10,000 batches, 5000 iterations) in GENEPOP 3.3 (Raymond and Rousset, 1995). The hierarchical Bayesian approach implemented in BAYESCAN (Foll and Gaggiotti, 2008) was used to test for signals of selection at the loci. Genetic differentiation among samples was quantified by global and pairwise FST values, with associated significances evaluated by 10,000 permutations using FSTAT. Pairwise FST values were also calculated incorporating the null allele correction method implemented in FreeNA (Chapuis and Estoup 2006). Isolation by distance effects were tested by assessing correlation between both sets of pairwise FST values (as well as their corresponding linearised [FST / (1- FST)] (Rousset, 1997) values and the corresponding shortest sea distance between pairs of samples using Mantel tests with 10,000 permutations in GENALEX. Genetic structuring was also investigated using the Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to assess the most probable number of genetically distinct groups (K) represented by the data without a priori sample information. Each run had a burn-in of 100,000 Markov Chain Monte Carlo samples followed by 1,000,000 MCMC repetitions. Simulations were run 3 times for each proposed value of K (Aitken and Whitlock, 2013; Anderson, 2001; Angert et al., 2011; Assis et al., 2018; Assis et al., 2017) to assess convergence with optimal models inferred using ΔK (Evanno et al., 2005). A PCoA of pairwise genotypic distances between individuals was performed using the methods implemented in GENELAEX 6.5 (Peakall and Smouse, 2006).

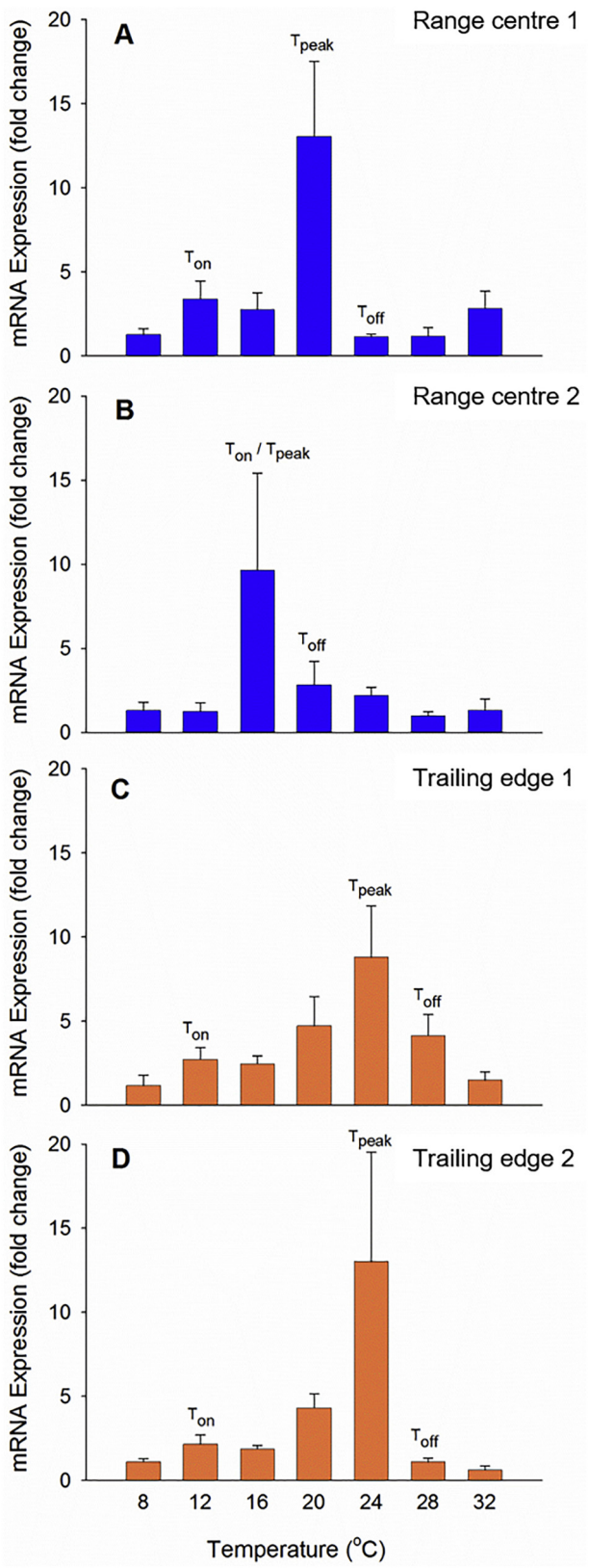

Fig. 2. Mean (± 1 S.E.) relative expression of *hsp70* in *Laminaria digitata* from range centre (A + B) and trailing edge locations (C + D) to acute 1 h immersed temperature shocks in April 2015. Relative expression calculated against two housekeeping genes Rubisco and 18srRNA. $n = 5$ per temperature treatment.

3. Results

3.1. Heat shock experiment

The HSR of all experimental trials followed a clear pattern of up-regulation; relative expression of *hsp70* increased with increasing temperature until a threshold was reached and a marked decline was subsequently observed. As such, thermal set points of the HSR (T_{on}, T_{peak} and T_{off}) could readily be identified from the plotted data (Fig. 2).

3.1.1. Regional comparison (spring sampling only)

When all four populations were compared in spring, there was a significant interaction between region and temperature (Pseudo-$F_{6,12} = 3.03$ $p = .04$; Table S2). Upregulation of *hsp70* occurred at higher temperatures at the trailing edge compared to range centre populations with this pattern consistent between sites within regions. Overall, magnitudes of expression were similar between populations with maximal upregulation ranging from 8.8 (± 3.05 S.E) – 13.06 (± 4.48 S.E) compared to control values.

T_{on} ranged from 12 to 16 °C with no discernible link to population origin, however, T_{peak} and T_{off} varied between regions occurring at lower temperatures in range centre populations (range centre 1 T_{peak} 20 °C T_{off} 24 °C range centre 2 T_{peak} 16 °C T_{off} 20 °C) compared to trailing edge populations (trailing edge 1 and 2; T_{peak} 24 °C T_{off} 28 °C) (Fig. 2).

3.1.2. Seasonal comparison (spring and autumn sampling)

Thermal assays were conducted in both spring and autumn for range centre 1 and trailing edge 1 to determine whether regional patterns were consistent across seasons. Here, there was no significant interaction between temperature, region and season, indicating that expression of *hsp70* was similar across seasons, in both regions (Pseudo-$F_{4,80} = 0.25$, $p = .92$; Table S3) (Fig. 3). However, there was a significant interaction between temperature and region (Pseudo-$F_{4,80} = 4.7$, $p = .001$; Table S3), with *hsp70* upregulation of trailing edge 1 occurring at higher temperatures compared to range centre 1, reinforcing the pattern observed when solely considering sampling undertaken in spring (Fig. 2).

T_{peak} and T_{off} remained fixed for both regions irrespective of sampling season (range centre 1 -T_{peak} 20 & T_{off} 24 °C; trailing edge 1 - T_{peak} 24 & T_{off} 28 °C) but a difference in T_{on} was observed. The HSR in summer was not evident until experimental temperatures were elevated to 16 °C at range centre 1 and 24 °C at trailing edge 1, representing an increase in $T_{(on)}$ of 4 and 12 °C for range centre 1 and trailing edge 1, respectively (Fig. 3).

3.2. Population genetics

The total number of alleles per locus range from 5 to 28 (mean = 14.3). Basic summary statistics for each sample are reported in Table S4 and showed a general trend of higher variability (NA, AR, HE and HO) among the two range centre sites compared to trailing edge sites. 18 out of 48 locus/sample tests of HWE revealed significant results, in all cases due to heterozygote deficits. With the exception of locus LD158, no locus exhibited significant heterozygote deficiencies at all four sites. Global FST was highly significant (FST = 0.181, $p < .0001$). All pairwise FST (Table S5) values were significant and revealed a clear hierarchical pattern wherein (i) the range centre and trailing edge populations were highly differentiated and (ii) comparisons between the two trailing edge populations yielded much higher pairwise FST values than between the two range centre populations despite the greater geographic distance between the range centre sites. This hierarchical structure was also evident from the clustering analysis for which ΔK supported K = 2 (Fig. 4) wherein the range centre and trailing edge samples were partitioned into two distinct clusters, while at K = 4 individuals robustly assigned to distinct clusters according to site.

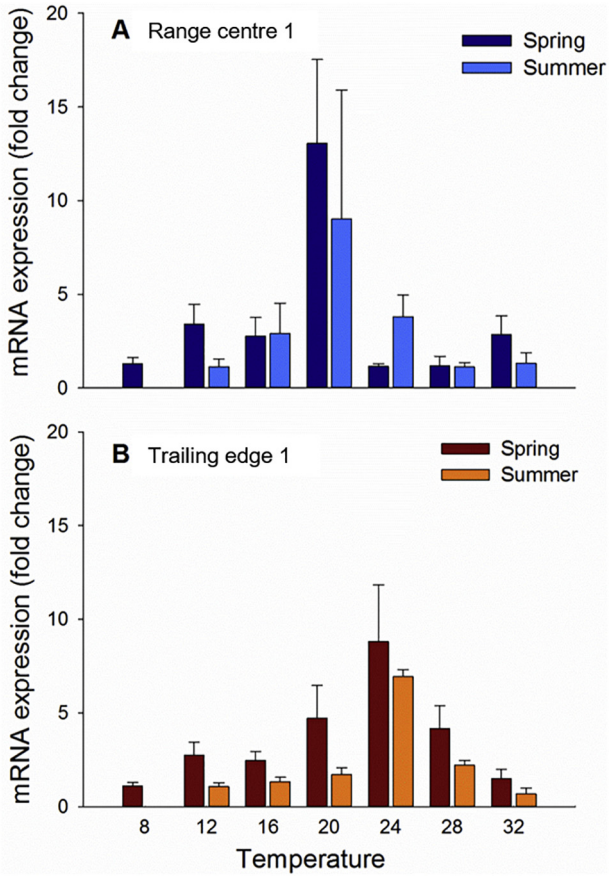

Fig. 3. Seasonal comparison of mean (± 1 S.E.) relative expression of *hsp70* in *Laminaria digitata* from range centre (A) and trailing edge locations (B) to acute 1 h immersed temperature shocks in April and September 2015. Relative expression calculated against two housekeeping genes Rubisco and 18srRNA. n = 5 per temperature treatment.

4. Discussion

Here, we provide three lines of evidence that taken together, suggests the presence of locally adapted thermal ecotypes, in the kelp *Laminaria digitata*, at trailing edge and range centre sites of its distribution. Firstly, we show clear differentiation in *L. digitata*'s Heat Shock Response, with the thermal set points T_{peak} and T_{off} higher in trailing edge compared to range centre populations. Secondly, this pattern was mirrored across the warmest and coolest times of year, indicating that differences were fixed regardless of previous thermal history. Finally, our microsatellite data revealed little gene flow between populations, meaning dispersal is far exceeded by the selection gradient, and as such, local adaptation is likely favoured over plasticity. The existence of these ecotypes may have serious implications for our understanding of thermal safety margins and *L. digitata*'s potential vulnerability to warming. Unlike many macrophyte species, *L. digitata* has not seen extensive population loss due to ongoing climate change but poleward range contractions are predicted over the coming century (Raybaud et al., 2013; Khan et al., 2018). As ecotypes possess a narrower thermal tolerance range than that of the overall species, thermal limits may be exceeded throughout *L. digitata's* range and not simply at its trailing edge. Moreover, *L. digitata*'s low dispersal capacity may mean that the ability of ecotypes to track their niche in space is undermined (Assis et al., 2017) which in turn could undermine recovery (Smale and Wernberg, 2013).

The presence of local thermal ecotypes raises concerns for the

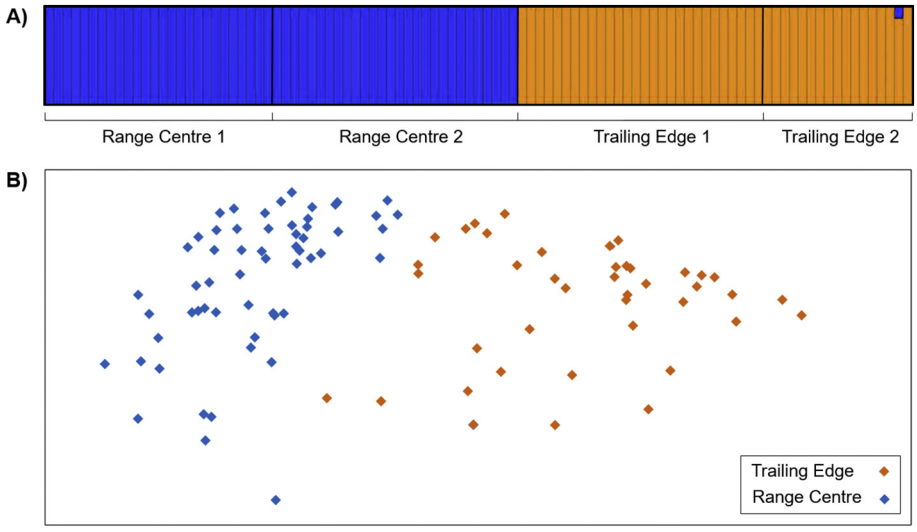

Fig. 4. A Genetic subdivision of *Laminaria digitata* based on STRUCTURE analysis. K1–4 were explored and K = 2 represents the most likely number of clusters based in ΔK outlier identification. B - Principal Coordinate analysis of pairwise genotypic distances between individuals. Individuals are coloured according to their sampling location (i.e. trailing edge or range centre).

effective use of current tools to predict species responses to future warming. Environmental Niche Models (ENMs) currently represent the most utilised tool to predict future species distributions under different climate scenarios. However, they either base thermal niches on the temperatures experienced by the species as a whole or source physiological data from a single climatic location (e.g. Sunday et al., 2012). Thus, they fail to incorporate any intraspecific variation in thermal niche that could increase the complexity and predictability of species responses (Harte et al., 2004; Thomas et al., 2004; Angert et al., 2011; Hällfors et al., 2016; Peterson et al., 2018). So far, ENMs for macrophytes (e.g. Khan et al., 2018), including *L. digitata* (Raybaud et al., 2013; Assis et al., 2018), have taken a correlative approach, and as such, may perform poorly where local ecotypes are present (King et al., 2018a). In order to rectify this and effectively calibrate ENMs, the relationship between ecotypes and temperature (genotype-by-environment interactions) needs to be characterised. This has been successfully achieved in terrestrial forests (O'Neill et al., 2008; Pearman et al., 2010; Doney et al., 2011) but has only been possible due to the availability of comprehensive historic provenance datasets (Matyas, 1996) that are distinctly lacking in other systems.

Identification of thermal ecotypes may also have valuable applications for future management of wild and farmed kelp populations. Transplantation of warm ecotypes to cooler regions may boost local resistance to warming and allow populations to track their shifting niche. Indeed, “assisted migration” is becoming an increasingly popular concept in terrestrial forest systems with policy frameworks already being developed (McLachlan et al., 2007; Aitken and Whitlock, 2013; Williams and Dumroese, 2013). Similar approaches are also being used in commercial seaweed aquaculture in East Asia to combat recent crop failures in the kelp, *Sacharina japonica* (Pang et al., 2007; Liu and Pang, 2010). *L. digitata* is harvested from wild populations as far north as Norway and introduction of warm tolerant ecotypes further north could ensure successful harvests in the future.

Our data also provide insight into the adaptability of trailing edge populations themselves. The greater differentiation between our trailing edge sites (despite closer proximity) and lower genetic diversity could be seen as indicative of a classic trailing edge, where populations have lower effective population sizes and are more fragmented (Eckert et al., 2008). Pearson et al. (2009) found that such factors resulted in trailing edge populations of the intertidal fucoid, *Fucus serratus*, being less tolerant to thermal shock than central populations i.e. reduced genetic diversity resulted in maladaptation. While our trailing edge populations did exhibit reduced genetic diversity, this did not prevent the formation of thermal ecotypes. It is likely that regional biogeographic contexts can account for this. While both *F. serratus* and *L. digitata* have similar range centre and trailing edge densities (King et al. *unpublished*; Pearson. *pers comm*), they differ in their overall extent and isolation from central populations. *F. serratus* occupies a small geographic area at its trailing edge, which is isolated from the larger central population by a lack of suitable habitat in the Bay of Biscay. In contrast, no such geographic barriers exist for *L. digitata* and it is contiguously distributed around the rocky reefs of the western coastline of the UK. As such, differences in isolation time, population connectivity and fragmentation may underpin variability in response patterns between these two species.

One aspect of the HSR that exhibited plasticity was T_{on} that increased in the autumn sampling period by up to 12 °C. Plasticity in T_{on} has been commonly documented in the literature (see Barua and Heckathorn, 2004) and is thought to allow fine tuning of the HSR to a highly variable environment, avoiding costly over production (Hochochka and Somero, 2002), which can decrease fitness (Feder et al., 1992; Krebs and Loeschcke, 1994). The cellular basis of this plasticity via the regulation of the transcription factor, heatshock factor-1 (*Hsf1*) which regulates expression of HSP genes (Voellmy, 1996) in what is known as the ‘cellular thermometer’ model (Craig and Gross, 1991). Under non-stressful conditions several HSP's bind to *Hsf-1* supressing HSP transcription. When conditions become stressful, HSP's dissociate from *Hsf-1* to perform their chaperoning roles (Parsell and Lindquist, 1993) and thus *Hsf-1* is free to induce transcription of HSP's. Increased incubation temperature and heat shocks increase the number of gene induction events which raises the standing level of HSP's in the cell (Maloyan et al., 1999). This increased standing stock can maintain protein refolding without dissociation of HSP's from the *Hsf-1* and so induction will occur at higher temperatures. At the end of summer, when kelps may have been subjected to multiple heat shock events, standing stocks will be at their greatest and thus responsible for an increase in T_{on}.

5. Conclusion

Our study provides evidence for local adaptation in an important kelp, which has implications for our understanding of thermal safety margins and how this species may respond to warming. King et al. (2018b) recently found intraspecific variation in thermal tolerance and restrictive dispersal to be common traits in marine macrophytes (seaweeds and seagrasses). Therefore, local adaptation may be a common trait for kelp and seaweeds in general. Indeed, transplants that are the most direct method to detect local adaptation (Merilä and Hendry, 2014) have clearly demonstrated distinct physiological differences between native vs transplanted populations (Gerard and Du Bois, 1988;

Saada et al., 2016). However, further experimentation on the progeny of assumed ecotypes is still required to exclude any effects from transgenerational plasticity that may mimic or obscure patterns of adaptation.

Quantifying what the presence of ecotypes means for the climate driven redistribution of *L. digitata,* and other macrophytes, is currently constrained by a lack of fine scale empirical data. The research effort required to attain the resolution needed to produce bespoke calibrated ENMs is clearly unfeasible. Therefore, a first step should be to gather the relevant data in a few ecologically representative species, such as *L. digitata*. In this way, it may be possible to make generalisations across phylogenies and allow a more precise understanding of thermal safety margins to be attained and areas of greatest vulnerability to be identified.

Declaration of interest

The authors of the study declare that there is no conflict of interest.

Acknowledgments

We thank all those who helped during field collection periods at the Scottish Association for Marine Sciences, Herriot Watt University Orkney campus and the Marine Biological Association. N.G.K. was funded by an Institute of Biological, Environmental and Rural Sciences PhD studentship. Funding for P.J.M. and N.G.K. was provided through a Marie Curie Career Integration Grant (PCIG10-GA- 2011- 303685) and the Aberystwyth University Research Fund (1211Z-01). L.S.H. was funded through an Aberystwyth University Doctoral Career Development Scholarship. D.A.S. is supported by an Independent Research Fellowship award by the Natural Environment Research Council of the UK (NE/K008439/1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.03.004.

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